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THE RESISTIVITY OF MICROORGANISMS TO THERMAL INACTIVATION BY DRY HEAT

Final Report

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Experimental

I. Chamber

A number of types of chambers were investigated for determining the dry heat resistivity of microorganisms. As an initial approach to study the problem under more realistic conditions it was decided to construct a chamber which would be inserted within a precisely controlled oil bath. The chamber is illustrated in Figure 1 and is capable of maintaining the temperature in the oil bath within $\pm$ 0.1°C and preheating a gas to the desired temperature before being admitted into the chamber. The temperature in the bath is heated by two 1000 watt bayonet resistance heaters, the heat being distributed by two stirrers. In order to regulate the temperature the Dynapac 15 temperature control unit (Lab-Line Instruments, Inc., Melrose Park, Illinois) was used, the sensing device being a thermistor probe capable of a response to within 0.02°C.

The chamber itself is a 6 inch brass cylinder constructed in two sections and joined by flanges. A teflon gasket has been inserted between the two flanges to decrease vertical thermal conductivity along the walls. Air is admitted in the bottom of the chamber and enters the chamber through a second perforated disc. The lid of the chamber is constructed of 5 inches of asbestos which in turn is sealed along the sides with nylon tape and the bottom is

covered with a plastic disc. The lid has a larger disc placed at the top which facilitates removal and positions the lid in the chamber. It is constructed of masonite and does not come in direct contact with the sides of the chamber but is separated by two gaskets, one of rubber and one of teflon. All materials were cemented with epoxy resin.

The temperature was monitored with three copper-constant thermocouples connnected to either a Leeds and Northrup millivolt potentiometer or continuously monitored by a Bausch and Lomb VOM-7 recorder.

The samples were placed on a 5 inch perforated platform positioned 5 centimeters from the bottom of the chamber.

II. Characteristics of the Chamber

The rationale behind the construction of this chamber was to place the microorganisms on an inert support in an isothermal zone under defined conditions. It was hoped to be able to vary the drying rate, the composition of the atmosphere and vacuum. Come-up times would be minimized by bleeding preheated air into the chamber. It became apparent with experience that certain shortcomings were inherent with this chamber. These are:

1. Heating is predominantly by convection. The isothermal zone in this chamber extends about 15 cm. high without

forcing air through the chamber and approximately 20 cm. when air is passed through the chamber. For any definition of the events occurring during the exposure to dry heat this chamber will not be precise enough. (See proposed new chamber described below.)

2. Attempts to regulate the come-up time by passing preheated air through the chamber was successful but this process introduced another variable in that survival was decreased as a result.

3. To speak of temperature within a chamber is misleading for one must actually consider heat and events occurring in the vicinity of the cell. It is actually the air that is being heated, and for the achievement of an isothermal zone the thermal conductivity of the air, which is heated by the walls of the chamber, is a limiting factor. The net result is that equilibrium does not really become established. On a microscale, which is the spore on a surface, differences (the magnitude of which is difficult to assess) in thermal energy transfer occur.

4. The temperature control was very stable in a macro sense but as seen below there are other methods for controlling come-up time and of minimizing the volume of air in the chamber and artifacts which might arise from uncontrolled convection.

III. Microorganisms and Assay Procedure

Spores of Bacillus subtilus var niger was the test organism of choice which was used throughout these experiments. It should be noted that the ultimate objectives of this project are to determine the thermal resistivity of as wide a variety of microorganisms as possible and this phase will be initiated in the next test period. The procedures employed for assay are those described in a previous publication (Applied Microbiology 11: 202-210, 1963) with the exception that incubation was conducted at 30°C instead of 37°C for 16-24 hrs. In certain cases incubation was much longer, to check on any injured cells which grow more slowly. Prolonged incubation has not affected recovery in our experiments.

The support surfaces are of two kinds, glass fibre filters and the membrane type. The glass filters were also described in the above publication. The membrane filters presented greater difficulty. The characteristics which they had to possess were quantitation and retention of wetting powers after exposure to elevated temperatures. Of the many commercially available filters examined only two showed promise, Gelman GA-6 filter and S and S 2500. The non-uniformity of the Gelman 6A-6, coupled with its poor wettability after exposure to elevated temperatures caused the selection of S & S 2500 as the support of choice. S & S 2500 is satisfactoryin most respects. Its one deficiency

has been a tendency to allow the water to sweep the spores to one portion of the filter upon rehydration. Care in placing the filter on the agar has minimized this aspect.

Various procedures to verify quantitation have been employed, both for controls and for the filters exposed to dry heat. In all cases examined, no spores have been blown off the filters during heating, either under static conditions or when air is blown through the chamber.

On the glass filters approximately 2 to 3 x 10^6 spores/filter were used. Each filter was blended in chilled water for three minutes and after serial dilution, surface plated on tryptone-glucose-yeast extract agar.

For membrane filters the number of spores embedded onto a filter was selected for each interval of exposure so as to give, after exposure to heat, approximately 100 to 200 colonies/filter.

The organisms on a support surface were dried for $2\frac{1}{2}$ hours at 45 C and equilibrated overnight over silica gel. This is an arbitrary procedure. There is data that more drastic drying, although not decreasing the control filters to any great extent, decreases survival during exposure to dry heat. This aspect will be investigated in the future.

IV. Temperature

The temperature range employed has been 106 ± 0.25°C. The oil bath was generally 0.5°C higher than the chamber. The temperature within the chamber varied within the range stated above. Attempts to stabilize the temperature in an absolute sense were only partially successful even though the unit ran continuously. One factor of extreme importance was the fact that the entire lid had to be removed in order to insert the perforated tray upon which the samples had been placed. This introduced ambient air into the chamber and accounted for a lag in the reestablishment of the desired temperature. Redesign of the lid so that a number of different samples could be introduced at different intervals was not successful since this necessitated the use of a plunger-type of action to seal the system and the sharp introduction of new air which upset the equilibrium greatly and for a long interval. The most practical and efficient method was therefore to lift the entire lid off gently and place the samples in the chamber at the same level. Vertical stacking interfered with the establishment of an isothermal zone of any appreciable depth. Analysis of the temperature profiles indicated strong interference with normal convection patterns and large temperature differences of 2°C between adjacent levels.

V. Results

The results of representative experiments are presented in Figure 2. which illustrates many of the factors initially considered. In comparing glass fibre filters to Gelman GA-6 membrane tri-acetate filters it is noted that the glass filters (curve 1) were more consistent, and that their average value was equivalent to approximately a one log cycle decrease after four hours of exposure at 106°C. The normal come-up time and temperature relationship was characteristically asymptotic. Within four minutes after the insertion of the samples, which took on an average of 25 seconds, the air temperature within the chamber was within one degree of the final chamber temperature and seven to eight minutes within 0.25°C or less. Attempts to shorten the come-up time, in anticipation of the use of higher temperatures, led to the interesting observation that an additional decrease in survival occurred after four hours. This occurred in all of a number of experiments designed to evaluate this factor and varied from an additional logarithmic to almost two logarithmic cycle decrease in survival. Curve 2 in Figure 2 illustrates this observation using 10 minutes of preheated air obtained from a bench-top outlet and 230 minutes of normal convection heating. The experiment for curve 3 differed in that the air used for preheating was dried by passage through a dry-ice

trap. These experiments indicate the complexity of the events occurring during air drying and that experiments will have to be conducted to analyze thermal energy transfer. A certain number of experiments were also conducted to measure the effect of predrying of the spores on subsequent survival within the chamber. As the spores were dried more severely, survival decreased somewhat.

Two other points should be made. In Figure 2 filters removed after 10 minutes of exposure to forced and preheated air showed the anticipated decrease in spore population and that four hours of forced air at ambient temperature did not result in the loss of spores from the filter by physical removal.

The lines drawn in Figure 2 are essentially hypothetical since only the four hour values are considered and are only illustrative in nature. More complete data is presented in Figure 3 below.

The data in Figure 3 represents at least 2 duplicate experiments for each value with five replicates for each experiment. The values used for plotting the curve were the averages and the range of replicates and are plotted at each sampling period. Wider variations are experienced

as the exposure time is increased, a characteristic of survivor curves in general. The control filters not exposed to heat, were in extremely close agreement with each other. The membrane filter technique required a greater number of organisms to be placed on a filter as the exposure time is increased. The variation will therefore be expected to be greater until most of the variables which can affect viability are understood and controlled. It is hoped that modifications in the chamber will minimize this variation.

It is seen in Figure 3 that the curve is essentially linear except at exposure times of two hours or less. A projection of the line does not pass through the origin. A one log cycle decrease in the linear portion of the curve occurs after approximately 4 hours and 20 minutes. A more precise analysis of the thermal inactivation curve will be presented in the next report in comparison with the results at other temperatures. In order to detect whether or not any spores are removed during the drying cycle by convection currents test filters were covered with a sterile filter and held together by clips during exposure. No increased recovery of spores was detected in these experiments.

VI. Future Experiments

1. Thermal death curves with B. subtilis var niger will be continued for comparison with data obtained by other investigators and so as to verify the data obtained from the test chambers.

2. The thermal resistivity of isolates from a variety of sources will be obtained.

3. A new chamber will be constructed in which the extent of convection heating will be minimized. This will be accomplished by the use of copper plates relatively close together, heated by thin resistant heaters, and surrounded with proper insulation. The samples will be introduced on grids between the plates. The system will be capable of being sealed so that the atmosphere surrounding the samples can be controlled. The thermal effects of air, nitrogen, and helium will be compared and it is anticipated, an insight obtained to evaluate the importance of thermal conductivity. The come-up time should be appreciably shortened.

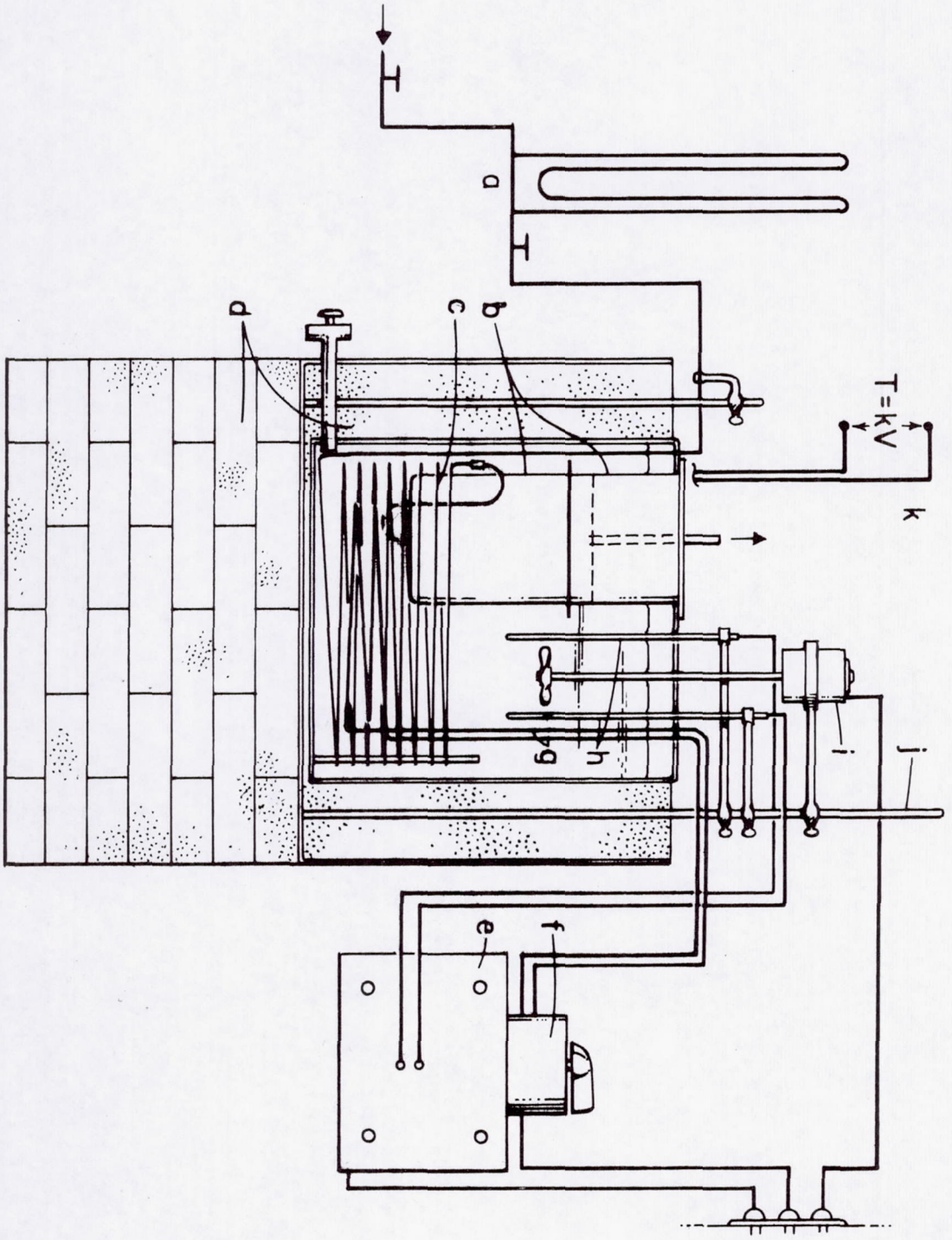
4. Spores will be equilibrated at various relative humidities before and after exposure to thermal energy so as to investigate two diverse factors: (1) the importance of the extent of dehydration on resistivity and (2) any possible modification in recovery by controlled rehydration.

Other experiments will be continued whereupon spores will be predried for longer periods in an oven and/or over desiccant prior to insertion in the test chamber.

Figure 1. Heating Chamber

Key

a. Manometer
b. Experimental Chamber
c. Copper Tube Coil
d. Heat Insulation
e. Temperature Controller
f. Powerstat
g. Heating Coil
h. Heating Knives
i. Stand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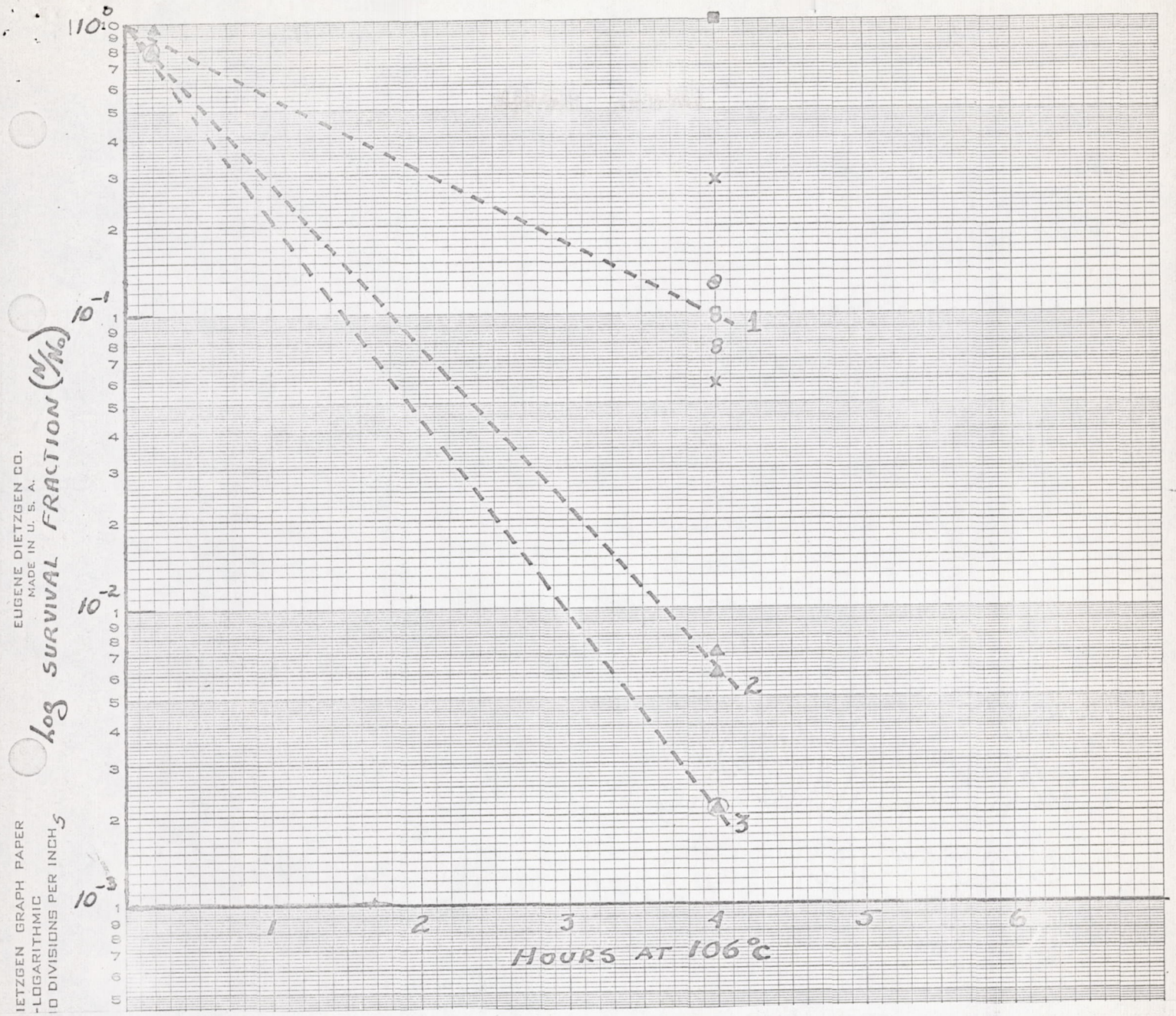

Figure 2. Thermal inactivation of Bacillus subtilis var. niger at 106.3°C.

Glass Fibre Filters

- ● Normal exposure at 106°C.
- ▲ 10 minutes exposure to forced circulation followed by 230 minutes of normal exposure at 106°C.
- ◉ Same as ▲ but dried air employed.
- ■ Forced air for 240 minutes at ambient temperature (20°C).

Gelman Filters

- ✕ Normal exposure at 106°C.

Figure 3. The survival fraction vs. time curve of *Bacillus subtilis* var *niger* at 106 ± 0.25°C.

NO. 340-L510 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH
EUGENE DIETZGEN CO.
MADE IN U. S. A.
SURVIVAL FRACTION (N/N₀)
TIME (HOURS)
4
8
12
16
20
24

FIGURE 3.